

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: H. Lowenheim Attorney Docket No.: SOPH116953
Application No.: 09/622,719 Group Art Unit: 1635
Filed: October 18, 2000 Examiner: K.A. Lacourciere
Title: METHOD FOR THE TREATMENT OF DISEASES OR DISORDERS OF
THE INNER EAR

THIRD DECLARATION OF JONATHAN KIL

Seattle, Washington 98101

January 4, 1904

TO THE COMMISSIONER FOR PATENTS:

I, Jonathan Kil, declare as follows:

1. I am the Chief Executive Officer of Sound Pharmaceuticals, Inc., Seattle, Washington, and I am familiar with the subject matter disclosed and claimed in the above-identified application.

2. My colleagues and I conducted the following experiments to assess the effect of a variety of antisense oligonucleotides on the level of expression of p27^{Kip1} mRNA in mouse NIH 3T3 cells cultured *in vitro*.

3. NIH 3T3 cells were transfected with the 14 antisense oligonucleotides shown in Table 1. The antisense oligonucleotides corresponded to portions of the target p27^{Kip1} mRNA. The location of each oligonucleotide is given with reference to the sequence of the mouse p27^{Kip1} cDNA (GenBank accession number U09968; reported in Polyak, K., et al, *Cell* **78**: 56-66 (1994)). Antisense oligonucleotides SPI5114 and SPI5116 have identical nucleic acid sequences, but SPI5116 includes both phosphothioate and 2' MOE backbone linkages.

Table 1

Oligonucleotide Name	Oligonucleotide Sequence	Location of Oligonucleotide
SPI5101	TGGCTCTCCTGCGCC	306-320

Attachment A

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS^{PLLC}
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

Oligonucleotide Name	Oligonucleotide Sequence	Location of Oligonucleotide
SPI5108	CATCCTGGCTCTCCTGCGCCAGCAC	301-325
SPI5114	CCGCTGACATCCTGGCTCTCCTGCG	308-332
SPI5116	CCGCTGACATCCTGGCTCTCCTGCG	308-332
SPI5906	TCTCACGTTTGACAT	1-15
SPI5907	ATTCCAATTGCGCTG	169-183
SPI5908	TCTCCACCTCCTGCC	227-241
SPI5501	TGCTCCGCTAACCC	247-440
SPI5505	GACACTGCTCCGCTAACCCAGCCTG	421-445
SPI5517	GACACTGCTCCGCTAACCCAG	425-445
SPI5518	CTGCTCCGCTAACCCAGCCTG	421-441
SPI5519	CACTGCTCCGCTAACCCAGCC	423-443
SPI5801	CATCCGCTCCAGGCT	34-48
SPI5806	CGTCCATCCGCTCCAGGCTCG	32-52

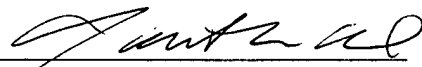
The cells were incubated in the presence of the oligonucleotide for 26 hours. Real time RT-PCR was used to measure the amount of p27^{Kip1} mRNA present in total RNA extracted from the treated cells.

4. Enclosed herewith as Attachment B is a graph showing the level of p27^{Kip1} mRNA in the cells treated with the different oligonucleotides, compared to the control level of p27^{Kip1} mRNA in cells treated with the Lipofectamine lipid delivery vehicle without oligonucleotides. The results shown in the graph demonstrate that all of the tested oligonucleotides caused a significant reduction in the level of p27^{Kip1} mRNA in the treated cells.

5. All statements made herein and of my own knowledge are true, and all statements made on information and belief are believed to be true; and further, these statements were made

with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

Respectfully submitted,


Jonathan Kil, M.D.

Date: 12/27/04

BFM:tm

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS^{PLLC}
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

Antisense Oligonucleotide Mediated Reduction in p27^{kip1} mRNA Level

